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PB-252 717-T

CONTRIBUTION TO THE STUDY OF THE IMMUNOLOGICAL ASPECTS OF CHRONIC BERYLLIOSIS

ENVIRONMENTAL PROTECTION AGENCY

1971

141029

PB 252 717-T

TR 318-73

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[Contribution à l'étude des aspects immunologiques de la béryllíose chronique]

Lille Médical, 16, No. 5, 1971, pp. 680-686.

Translated for the
ENVIRONMENTAL PROTECTION AGENCY
by
Translation Consultants, Ltd., Arlington, Virginia

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

BIBLIOGRAPHIC DATA SHEET	1. Report No. EPA TR73-318	2.	3. Recipient's Accession No.
4. Title and Subtitle Contribution to the study of the immunological aspects of chronic berylliosis			5. Report Date 1971 6.
7. Author(s) W. Van Ganse, J. Oleffe, W. Van Hove, G. Grdeten Briel			8. Performing Organization Rept. No.
9. Performing Organization Name and Address EPA ISD Translation Services Division Library RTP, NC 27711			10. Project/Task/Work Unit No. 11. Contract/Grant No.
12. Sponsoring Organization Name and Address Translated from the French by EPA			13. Type of Report & Period Covered 14.

15. Supplementary Notes

Lille Medical 16(5):680-686 1971

16. Abstracts

17. Key Words and Document Analysis. 17a. Descriptors

EPA ISD
Berylliosis
Beryllium

17b. Identifiers/Open-Ended Terms

Industrial Medicine
Beryllium poisoning-symptoms
Beryllium poisoning - diagnosis

17c. COSATI Field/Group 6-T 57-Y

PRICES SUBJECT TO CHANGE

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Exclusive permission granted to NTIS for the storage and distribution of this material/ 1.	UNCLASSIFIED	17
	20. Security Class (This Page) UNCLASSIFIED	22. Price 3.50

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CONTRIBUTION TO THE STUDY OF THE IMMUNOLOGICAL ASPECTS OF CHRONIC BERYLLIOSIS

The following tests were carried out on a 45 year old patient afflicted with chronic berylliosis:

(1) patch test with beryllium fluoride which was positive upon inspection beginning from a concentration of 0.1% and negative in the case of fifteen control subjects. A histological examination revealed the presence of lymphocyte infiltrates;

(2) normal lymphocyte transformation (79%) with phytohemagglutin as well as a discrete one using contact with (small) doses of beryllium (9%) and beryllium fluoride (12%) whereas higher doses did not cause lymphocyte transformation and it was found that the lymphocytes of a control subject experienced stimulation with any of the doses used;

(3) a dosage of immunoglobulins gave normal readings with the exception of a slight increase of the IgG;

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* Funds for Occupational Diseases, Brussels, and the Health and Social Medicine Laboratory (Prof. Dr. K. Vuylsteek) of the University of Gand.

** Funds for Occupational Disease, Brussels and the Department of Dermatology (Prof. Dr. G. Achten) of the St. Pierre University Hospital at Brussels.

*** Service of Internal Medicine, Departments of Endocrinology, Hematology and the Diseases of Metabolism (Prof. Dr. A. Vermeulen) of the University of Gand.

**** Funds for Occupational Diseases, Brussels.

(4) intradermal tests of Mantoux, Kveim as well as tests of house dust were all negative. This data appeared to confirm that tissual reactions induced by beryllium are caused by an immunological reaction of the "delayed hypersensitivity" type owing to the action of sensitized lymphocytes.

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Introduction

Although chronic berylliosis is a rare disease in Europe it should become more widely known for the following two reasons.

First of all, the recent applications of beryllium in the aeronautical and space industry (especially in the manufacture of rocket nose cones and gyroscopes) appear to support the possibility of a reappearance of this disease which had practically disappeared since around 1950 when the use of beryllium oxide ceased in the manufacture of fluorescent tubes.

Then, and above all, there is the surprising similarity to the disease sarcoidosis which is much more common but whose etiology is still unknown. This similarity suggests that the study of clinical and experimental lesions caused by beryllium could have general implications going beyond the mere scope of the toxicity of this metal.

The present work will attempt to arrive at specific conclusions relating to the pathogeny of chronic berylliosis. The clinical aspects of the patient on whom the tests were performed are used as a basis for this study. Some health and industrial characteristics previously described (Groetenbriel, Van Ganse and Oleffe, 1970) will only be summarized very briefly here.

Clinical observation

In 1946, at the age of 21 years, a laboratory technician participated in the manufacture of fluorescent tubes using as a base a powder composed of beryllium oxide, inorganic phosphates, tungsten and arsenic. No precaution was taken to prevent inhalation of this powder whose use was eventually given up after a period of 4 years.

It was not until 1966 that the patient experienced a feeling of respiratory difficulty and became more readily out of breath. Although a clinical examination turned up no abnormality, radiology (Figure 1) evoked the possibility of chronic berylliosis. The functional respiratory examination (Table 1) showed a moderate reduction of pulmonary volumes without signs of bronchoconstriction. There is a slight reduction in CO transfer and the arterial gazometry at rest and with stress is normal.

A patch test with beryllium fluoride was positive at concentrations of 2%, 1% and 0.1%.

The patient was seen on a regular basis from 1966 to June 1970. On the whole, the clinical, radiological and functional prognoses underwent no appreciable modification in the absence of any treatment.

[caption for Figure 1].

Figure 1. Cutaneous biopsy carried out 48 hours after the patch test using a concentration of 0.01% of beryllium fluoride.

The presence of a histiolymphocyte flow into the dermis. The infiltrate tends to invade the deep layers of the epidermis with appearance of a discrete spongiosis*.

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* Figure 1, page 681 of original, not reproducible.

Table 1. Functional respiratory tests*

	February 1966	December 1968	June 1970
Static volumes			
Vital Capacity (VC)			
(l. BTPS)	3.975 (89% NI)	3.560	3.390
Total pulm. vol.			
(l. BTPS)	1.505 (83% NI)	1.745	1.250
Dynamic volumes			
Forced expiratory vol. for 1 sec			
(l. BTPS)	3.100 (89% NI)	2.460	2.340
Transfer of CO**			
(ml/min/mm Hg)	18.3 (52% NI)	20.2	21.0
Gas in the arterial blood			
(a) rest			
$PaCO_2$ (mm Hg)	36		38
$PaCO_2$ (mm Hg)	78		74
pH	7.40		7.38
stand, HCO_3 (meq/L)	22		22
(b) stress (100 Watt)			
$PaCO_2$ (mm Hg)	27		
PaO_2	80		
pH	7.35		
stand, HCO_3 (meq/L)	17		

* Standard values in: Cotes, J. E., Lung Function, 1 vol. 541 p, Blackwell Scientific Publications, Oxford 1965 (pp. 338-356).

** With apnea.

Specific Tests: Technique and Findings

1. Patch test

Epicutaneous tests were carried out with beryllium fluoride in aqueous solution and with a vaseline-base excipient. Readings were made after 72 hours. The reactions were clearly positive beginning with a concentration of 0.1%. The histological examination of the reaction with 0.01% revealed

the presence of a discrete histio-lymphocyte infiltrate (Figure 2). With this same concentration, investigation of mucopolysaccharid acids (toluidine blue, alcian and hale blue) showed no special deposits in either the dermis or epidermis. It was, nevertheless, found that the Bizzozero nodules appeared more accentuated with hale blue.

[caption for Figure 2].

Figure 2. Standard photo showing the presence of a quite dense bed of micronodules in both pulmonary fields. Tomographies of the hila and axillary regions (confluence?) did not show any abnormalities*.

2. Dosage of immunoglobulins

The immunoglobulins IgG, IgA and IgM were dosed according to the Wieme and Veys method (1970) with the following results:

IgG 2300 mg% (nl. 860 to 2000)

IgA 310 mg% (nl. 260 to 770)

IgM 80 mg% (nl. 15 to 388).

3. Investigation for humoral antibodies

The rate of O antistreptolysins was 12 units. Since the Widal reaction was negative (the patient had never been vaccinated), an antityphus vaccination was carried out using 0.5 ml of typhus and paratyphus vaccine (TABC, 1 billion germs/ml). After three weeks the Widal reaction was still negative.

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* Figure 2, page 682 of original, not reproducible.

4. Lymphocyte transformation

Cellular immunity was studied in vitro by culturing lymphocytes in contact with phytohemagglutinin (PHA), pure beryllium and beryllium fluoride. Five hundred cells were counted after periods of incubation amounting to 72 and 96 hours. The culture of the PHA gave normal results: 79% of the lymphocytes of the patient underwent a blastoid transformation which is comparable to the 72% obtained with a control subject. However, in contact with pure beryllium (0.357 mg + 1 ml plasma + 2 ml TC 199) and beryllium fluoride (0.33 mg + 1 ml plasma + 2 ml TC 199), the lymphocytes of the control subject underwent no transformation whereas respectively 9% and 12% of the lymphocytes of the patient were transformed.

This percentage was reached after 72 hours and did not increase subsequently.

Upon contact with a higher concentration (0.476 mg Be + 0.400 mg BeF_2), the percentage of blastoid elements was reduced respectively to 1% and 4%. We did not test lower concentrations.

A reaction of von Pirquet and an intradermal reaction of Mantoux[1], using a 1% concentration, remained negative after 48 hours.

A Kveim test[2] was negative after three weeks.

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1. Pure beryllium fluoride, ref. No. 1178, Fluka A. G., Buchs S. G., Switzerland.
   Berna tuberculin, 1 ml = 100,000 IU.

2. Siltzbock - Kveim antigen, Commonwealth Serum Laboratories, Melbourne, Australia.

Intradermal tests using house dusts[3] at concentrations of 0.5 mg/ml, 0.05 mg/ml and 0.005 mg/ml were negative.

Other Examinations

As a result of recent American works (Kelley et al., 1969, Stoeckle et al., 1969) pointing out the frequency of some biochemical abnormalities in berylliosis, we carried out the following determinations which, to a one, provided a standard findings for our laboratory:

sedimentation rate of the erythrocytes: 8/22 mm/h;

uricemia: 6.3 mg %;

calciuria after 48 hours of a hypocaleic diet: 133 mg/24 hours;

calcemia: 10.8 mg %;

phosphoremia: 2.7 mg %;

alkaline phosphatases: 12.6 U.K.A.;

thymol: 3.0 U;

zinc sulfate: 4.9 U;

S.G.O.T.: 19 U;

S.G.P.T.: 30 U;

Rose test 4 U; S.S.K.P. 0, L.F.F. 20, L.K.P. 40;

antinuclear factor: negative.

Among the other routine biochemical examinations we shall use exclusively a free hyperlipidemia (cholesterol 423 mg %, total lipids 1003 mg %, β lipoproteins 352 U). An electrocardiagram showed disturbances in repolarization with an aspecific appearance in the anterolateral region.

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3. Huisschilfers, Diephuis Allergens, Groningen, Netherlands.

Discussion

Based on the vast experience of the Beryllium Case Registry in the United States (535 cases of chronic berylliosis recorded in 1966), Stoeckle et al. (1969) feel that this diagnosis can be used in conjunction with: (a) a past history of significant exposure and (b) specific clinical criteria. The latter are:

(1) presence of diffuse opaque areas on the radiographs of the thorax;

(2) functional syndrome chracterized by a ventilatory deficit of the restrictive type associated or not with a decrease in the diffusion capacity;

(3) histological image of chronic interstitial pulmonary inflammation, often of the granulomatous type;

(4) functional or morphological signs of poisoning in organs other than the lungs;

(5) presence of beryllium in the tissues.

They believe that the first two criteria are obligatory whereas the others are optional.

We believe that our patient fulfills the conditions required for the diagnosis of chronic berylliosis. Nevertheless, we are aware, as, furthermore, emphasized by Hardy (1962), that the use of the term berylliosis is probably incorrect since, contrary to the pneumoconioses, the pathological attack is not sited only at the pulmonary level but likewise often extends to the liver, spleen, lymphatic ganglia, kidneys and to the teguments. It

is also true that this disease is frequently accompanied by biochemical deteriorations e.g. hypercalciuria, hyperuricemia and that it can regress during a treatment to the corticosteroids.

Whereas it is probable that a mechanism of direct toxicity forms the basis for the acute symptoms occurring following exposure to high concentrations of beryllium, immunological processes appear to contribute to the genesis of chronic berylliosis.

Indeed, the insidious slow development of the disease (some latent periods amounting to as much as twenty-three years have been reported), the histological aspect of the lesions, the absence of relationship between the quantity of beryllium present in the tissues and the intensity or duration of symptoms (Lieben et al., 1963), the cases of favorable results from treatment with corticosteroids and, finally, the reproduction of characteristic pulmonary granulomatous lesions by intratracheal injection of beryllium oxide with the rat (Chiappino et al., 1969) suggest the concept in the pathogeny of this disease of a mechanism of hypersensitization of the "delayed hypersensitivity" type.

This hypothesis is not recent. As early as 1951, Curtis had clearly described the clinical and microscopic characteristics of the patch test for beryllium which he considered as compatible with an allergic etiology.

Patch tests carried out at 14-month intervals on this patient disclosed that he remained sensitized to BeF_2 and that his cutaneous reaction even became accentuated (Table 2).

For the purpose of making certain that a reaction of the allergic type was genuinely concerned and not just an orthoergic effect (by primary irritation), we carried out tests using very low concentrations with this patient at the same time comparing the results from tests performed with the same concentrations on the control subjects. As can be seen from Table 3, positive tests after 48 hours at concentrations less than 1% were not observed in the case of fifteen subjects free of pulmonary infections but affected either by an eczema with contact proven or by an eczema with an unknown etiology, or even by a non-eczematous dermatosis.

Referring to the situation observed within the scope of contact dermatoses, we desired particularly to perform on this patient a cutaneous test using a concentration - dilution with BeF_2 (Achten and Oleffe, 1966). Indeed, in the presence of a substance having a specific toxicity for the skin (as does BeF_2), only those reactions can be considered allergic which are observed at a much lower concentration than those close to toxicity. In the extreme, the presence alone of a positive histological reaction without any clinical reaction argues for the allergic origin of this reaction.

Table 2. Patch test with beryllium fluoride

	BeF_2 in vaseline excipient					BeF_2 in aqueous solution	
	0.01%	0.1%	1%	2%	5%	0.001%	0.01%
April 1969	-	+++	+++	+++	++++	not tested	not tested
June 1970	++	+++	+++	++++	not tested	±	++

- Reading after 72 hours: + = erythema; ++ = erythema and pimply reaction; +++ = free vesiculation (mini-eczema); ++++ = considerable vesiculation passing beyond the localized area of the test.

On the basis of the existence of a histological reaction of the allergic type found on bioptic samples made with this patient during tests made with very low concentrations of BeF_2 (which gave no reaction in the case of the control subjects), it can be concluded that we have here an especially valuable diagnostic tool.

In addition, although the patch test cannot be considered as an adequate pathognomonic tool, its histological study considerably increases its value. It makes up one important argument for an immunological pathogeny.

We were likewise able to find a slight increase in the IgG immunoglobulins as recently reported by Resnick et al. (1970). This increase is, nevertheless, not constant. It is missing in two of their six cases of chronic berylliosis. It should be noted in this respect that there is a similar lack of complete agreement insofar as concerns the rate of immunoglobulins in sarcoidosis. In some cases, we find the IgA to have higher rates (Buckley et al., 1966, Goldstein et al., 1969) and, in other cases, the IgG (Buckely and Dorsey, 1970) have the higher rates.

We believe that the semi-normal rate of immunoglobulins and the presence of O antistreptolysins coincide with the clinical and histological opinions which attribute the etiopathogeny of chronic berylliosis to mechanisms taking their origin from cellular rather than humoral immunity.

The absence of reaction to tuberculin, to the antigen of the Kveim test and to extracts of house dust in an isolated case of chronic berylliosis clearly is only one relative indication as to the presence of a cutaneous anergia. It is possible that other antigens could have caused positive

reactions. Nevertheless, this statement is in agreement with the observations of Lordon et al. (1968) in the case of patients affected by active sarcoidosis. They found a hyporeactive condition especially with respect to the antigens of Candida albicans and the mumps virus. This anergia probably even sometimes persists after the clinical recovery (Israel and Sones, 1965).

In order to study this hypothesis, we called upon the technique of transformation of leucocytes. We did not attempt to induce a sensitization to dinitrochlorobenzene since the latter occasionally caused violent reactions whose risk did not appear justified within the medical and legal context of our report.

A normal blastic transformation of the lymphocytes stimulated by the phytohemaglutinin was observed by Buckley et al. (1966) in seven patients attacked by non-evolutional sarcoidosis. This transformation was lacking in four other patients who showed exacerbations accompanied by a temperature and functional deterioration.

The normal reaction to phytohemagglutinin of the lymphocytes on the part of our patient showed the absence of changes in cellular immunity, at least in vitro. The reactions to the beryllium contact are more difficult to interpret. There appeared a blastoid transformation but the proportion of cells transformed remained low. The reduced rate of blastoid elements - corresponding to the late hypersensitivity noted in the epicutaneous tests - could correspond to a reduction in the number of lymphocytes stimulated by the antigen ("memory cells"). On the other hand, and here we have the hypothesis which we are most willing to use, it is likewise possible that the beryllium concentrations were still too

Table 3. Results of the patch test with beryllium fluoride on fifteen control subjects

Case	Test with BeF_2*					Observations	
	0,01 %	0,1 %	1 %	2 %	5 %	Reading after 48h	Reading after 72h
1. L.L.P. de Besnier.	—	—	—	—	—		×
2. H. eczéma des mains d'origine non déterminée.		—	+++	+++	+++	×	
3. D. eczéma des mains, ouvrier lithographe.		—	—	—	—	×	
4. L. boutons d'huile.	—	—	—	—	—	×	
5. M. eczéma par allergie au nickel.	—	—	—	—	+	×	
6. G. eczéma par allergie aux dérivés chromés.	—	—	—	—	++	×	
7. T. eczéma par allergie aux dérivés chromés.	—	—	—	—	—	×	
8. B. eczéma par allergie aux dérivés chromés.	—	—	—	—	+	×	
9. M. eczéma de cause non déterminée.	—	—	—	+	+	×	
10. N. eczéma de cause non déterminée.	—	—	—	—	—	×	
11. S. eczéma de cause non déterminée.	—	—	—	—	—	×	
12. R. eczéma des mains (allergie au nickel et au bichromate).	— +++	— +++	— +++	— +++	+++ ++++	×	×
13. L. eczéma des mains (allergie au bichromate).	—	—	—	—	—	×	
14** H. eczéma des mains (allergie au bichromate et au disulfure de tétraméthylthiurame).	—	—	—	—	—	×	
15. S. eczéma des mains (cause probable : ciment).	—	—	—	—	—	×	

Reading of the tests: cf. Table 2

* Excipient used = vaseline

** This patient had a delayed reaction for all concentrations 6 days after the tests.

Key:

1 - L.L.P. of Besnier; 2 - H. eczema of the hands of indeterminant origin; 3 - D. eczema of the hands, worker in lithography; 4 - L. oil pustules; 5 - M. eczema owing to a nickel allergy; 6 - G. eczema owing to an allergy to chromium derivatives; 7 - T. eczema owing to an allergy to chromium derivatives; 8 - B. eczema owing to an allergy to chromium derivatives;

[Key Continued]

9 - M. eczema from cause not determined; 10 - N. eczema from cause not determined; 11 - S. eczema from cause not determined; 12 - R. eczema of the hands (allergy to nickel and to the (nickel) bichromate); 13 - L. eczema of the hands (allergy to (nickel) bichromate); 14** - H. eczema of the hands (allergy to the (nickel) bichromate and tetramethylthiurame disulfide); 15 - S. eczema of the hands (probable cause: cement).

strong since an increase in these concentrations further reduced the number of transformations. This hypothesis should be tested by the use of very low concentrations of beryllium.

Acknowledgements

We should like to particularly express our gratitude to Professor Z. Eeckhaut (Laboratory of Inorganic Chemistry of Gand University) who was kind enough to supply us with a specimen of beryllium ore as well as to Doctor Matton (Cytogenetic Department of Professor J. Francois of Gand University) who helped us immeasurably in the tests of lymphocyte transformation. We are likewise grateful to Doctor Veys (Department of Physiotherapy of Professor H. Claessens of Gand University) who carried out the determination of the immunoglobulins.

Study performed at the Fund for Occupational Diseases at Brussels. (Official in charge was A. Dumont together with Medical Officer C. Groetenbriel) and read at the International Congress of Thoracic Pathology, Lille, 25-26 September 1970.

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